

**CLAIMS**

1. A physiological medium which comprises an aqueous solution in sterile purified water of:

- (i) a salt component comprising:
  - (a) from 100 to 150 mmol/L of sodium ions,
  - (b) from 2.5 to 6.2 mmol/L of potassium ions,
  - (c) from 0.1 to 2.5 mmol/L of calcium ions,
  - (d) from 0.4 to 25 mmol/L of magnesium ions, and
  - (e) from 96 to 126 mmol/L of chloride ions;
- (ii) a buffer component comprising
  - (f) from 21 to 27 mmol/L of bicarbonate ions, and
  - (g) from 1 to 12 mmol/L of TES, MOPS or BES;
- (iii) a substrate component comprising:
  - (h) 2 to 11 mmol/L of glucose
  - (i) 50 to 150  $\mu$ mol/L of glycerol and
  - (j) 7 to 15  $\mu$ mol/L of choline;
- (iv) an amino acid component comprising:
  - (k) 5 to 400  $\mu$ mol/L of glutamate
  - (l) 5 to 200  $\mu$ mol/L of aspartate and
  - (m) 100 to 2000  $\mu$ mol/L of glutamine;
- (v) a co-enzyme component comprising:
  - (n) 1 to 120 nmol/L of thiamine cocarboxylase;
- (vi) a vitaminoid component comprising:
  - (o) 40 to 70  $\mu$ mol/L of D- or DL- or L- carnitine;
- (vii) a protein component comprising:
  - (p) 5 to 200 m I.U./L of porcine or human insulin; and

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2. A physiological medium as claimed in Claim 1 which comprises
  - (viii) an antibiotic component comprising:
    - (q) 10 to 150 mg/L of chloramphenicol.
3. A physiological medium as claimed in Claim 1 or 2 wherein the salt component comprises:
  - (c) from 1.0 to 2.5 mmoles/L of calcium ions, and
  - (d) from 0.4 to 2.4 mmoles/L of magnesium ions.
4. A physiological medium as claimed in any of claims 1 to 3 wherein the salt component comprises  
135.32 mmoles /L of sodium ions, 5.00 mmoles /L of potassium ions, 1.25 mmoles /L of calcium ions, 0.45 mmoles /L of magnesium ions, as chloride salts, and 118.40 mmoles /L of chloride ions as sodium, potassium, calcium and magnesium salts.
5. A physiological medium as claimed in any one of Claims 1 to 4 wherein the buffer component comprises 25.00 mmoles /L of bicarbonate ions as sodium salt and 5.0 mmoles /L of N,N-bis (2-hydroxy ethyl)-2- amino-ethanesulfonic acid (BES).
6. A physiological medium as claimed in any of Claims 1 to 5 wherein the substrate component comprises 10 mmoles /L of D-glucose, 110  $\mu$ moles /L of glycerol and 10.0  $\mu$ moles /L of choline as the chloride salt.
7. A physiological medium claimed in any of Claims 1 to 6 wherein the amino acid component comprises 300  $\mu$ moles /L of L-glutamate as sodium salt, 20  $\mu$ moles /L of L-aspartate as sodium salt and 400  $\mu$ moles /L of L-glutamine.
8. A physiological medium claimed in any of Claims 1 to 7 wherein the co-enzyme component comprises 40.0 nmoles /L of thiamine as thiamine pyrophosphate chloride.
9. A physiological medium as claimed in any one of Claims 1 to 8 wherein the vitaminoid component comprises 50.0  $\mu$ moles /L of [-]- $\beta$ -hydroxy- $\gamma$ -trimethylaminobutyrate hydrochloride (L-carnitine).

10. A physiological medium as claimed in any one of Claims 1 to 9 wherein the protein component comprises 28.0 m. I.U./L of recombinant human insulin (expressed in E.coli).
11. A physiological medium as claimed in any one of Claims 1 to 10 wherein the antibiotic component comprises 100 mg/L of D-[-]-theo-2-dichloroacetamide-1-(p-nitrophenyl)-1,3-propane acid (chloramphenicol).
12. A method for producing a physiological medium according to any one of Claims 1 to 11 which comprises adding in the following order: sodium chloride, potassium chloride, calcium chloride, magnesium chloride, the TES, MOPS, or BES, thiamine, carnitine, choline, glycerol, insulin, aspartate, glucose, glutamate, glutamine, and sodium bicarbonate to sterile purified water, with constant stirring, making up to the desired volume, filtering and storing in sterile sealed vessels.
13. Concentrates for the preparation of a physiological medium as claimed in any one of Claims 1 to 11 which comprise the salt, buffer, substrate, amino acid, co-enzyme, vitaminoid and protein components, and dilutable with sterile purified water to form said physiological medium.
14. Concentrates for the preparation of a physiological medium as claimed in any one of Claims 1 to 11 which comprise the salt, buffer, substrate, amino acid, co-enzyme, vitaminoid and protein components, except for sodium bicarbonate, and dilutable with sterile purified water with the addition of sodium bicarbonate to form said physiological medium.